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# DIVISION S-7—NOTES

## LONG-TERM PATTERNS IN FOREST-FLOOR NITROGEN-15 NATURAL ABUNDANCE AT HUBBARD BROOK, NH

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### Abstract

To test the hypothesis that $\delta^{15}N$ in the forest floor remains constant over time, we measured $\delta^{15}N$ in forest-floor samples from 1969, 1978, 1987, and 1992 at the reference watershed, W6, at the Hubbard Brook Experimental Forest (HBEF), New Hampshire. The $\delta^{15}N$ of the Oa horizon increased significantly ($P < 0.05$) from 3.00‰ in 1969 to 4.89‰ in 1978, then decreased significantly to 3.81‰ in 1987 and remained near that level in 1992. In the Oie horizon, $\delta^{15}N$ increased significantly from 0.17‰ in 1969 to 0.91‰ in 1978 and remained at the higher level for the later years. Thus $\delta^{15}N$ was not at steady state in either the Oie or Oa horizon for the period 1969 to 1992 in the reference watershed. These data suggest that even relatively short-term disruptions of the N cycle (either by anthropogenic or natural disturbance) can alter the $\delta^{15}N$ in the forest floor, and should be considered in evaluating natural abundance data.

N ATURAL ABUNDANCE OF $^{15}N$ has been used to help evaluate N cycling and N losses (Johanisson and Högberg, 1994; Austin and Vitousek, 1998; Emmett et al., 1998), to compare plant species patterns of N uptake (Nadelhoffer et al., 1996), and to compare land-use history (Piccolo et al., 1994). An underlying assumption that soil $\delta^{15}N$ is at steady state is made in many studies, including laboratory experiments evaluating the change in soil $\delta^{15}N$ in response to mineralization and nitrification (Nadelhoffer and Fry, 1988), comparisons of a N deposition gradient in the NITREX study (Emmett et al., 1998), and models (Shearer et al., 1974; Hobbie et al., 1999). Although it is reasonable to assume that the

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$\delta^{15}N$ in the mineral soil changes very little over time because of the large pool size and long residence time, the steady-state assumption in the forest floor has not been established.

The stable N isotope ratio ($^{15}N$ to $^{14}N$) is useful in ecological research because it records the net effects of N transformations on the soil (Högberg, 1997). Microbially mediated processes discriminate against the heavier $^{15}N$, creating products that are depleted in $^{15}N$ and leaving the source pool enriched in $^{15}N$ (Mariotti et al., 1981; Shearer and Kohl, 1986). If the depleted product is exported from the soil (via uptake or leaching after nitrification or gaseous losses after denitrification), the remaining soil becomes enriched in $^{15}N$ (Létolle, 1980; Shearer and Kohl, 1986; Nadelhoffer and Fry, 1994). The fractionation during nitrification, ≈15 to 36‰ (Högberg, 1997), is significantly higher than that during mineralization (≈1‰; Högberg, 1997; Kendall, 1998).

Because litter $\delta^{15}N$ values are consistently lower than soil values, litterfall inputs tend to lower the $\delta^{15}N$ of the forest floor (Fry, 1991; Nadelhoffer and Fry, 1994; Högberg, 1997). Likewise, because soil is typically enriched relative to the atmosphere, N fixation also decreases soil $\delta^{15}N$. Nitrogen fixation incorporates atmospheric N, which has a $\delta^{15}N$ of 0‰, into plant material and subsequently into soil with a fractionation that typically ranges from −1 to +1‰ (Shearer and Kohl, 1986).

Other N fluxes may either deplete or enrich soil $^{15}N$; these include deposition and immobilization. The $\delta^{15}N$ of ammonium and nitrate in deposition varies considerably, the former from −14 to 9‰ (Hoering, 1957; Freyer, 1978; Paerl and Fogel, 1994), and the latter from −7 to 6‰ (Hübner, 1986; Garten, 1992; Kendall, 1998; Pardo et al., 1998). At the HBEF, the $\delta^{15}N$ of precipitation nitrate is −2‰ (Pardo, unpublished data, 1996–1998); $\delta^{15}N$ has not been measured for ammonium. Ammonium deposition may be more enriched (Hoering, 1957; Nadelhoffer et al., 1999) or less enriched (Freyer, 1978; Garten, 1992) in $\delta^{15}N$ than nitrate deposition. The effect of N deposition on forest soil, therefore, cannot be predicted a priori. At the HBEF, because nitrate deposition is depleted relative to soil (as is ammonium for the nearest site with data available; Nadelhoffer et al., 1999),

and given the small amount of deposition relative to the soil N pool, the net effect of N deposition would probably be a negligible decrease in soil $\delta^{15}N$. Few studies have evaluated the $^{15}N$ fractionation that occurs during N immobilization in the forest floor. Reported values for fractionation during abiotic and biotic immobilization are <2‰ (Nadelhoffer and Fry, 1994).

To evaluate whether $\delta^{15}N$ is at steady state in the forest floor, we obtained archived forest-floor samples from four sampling dates between 1969 and 1992 at the HBEF. The forest floor of a mature second-growth forest is described as being at steady state—a condition in which N inputs are balanced by N outputs (Covington, 1981). Measurements of the forest-floor N pool at the HBEF in 1977 and 1987 demonstrated no detectable change in pool size over that period (Woontner, 1990). We hypothesized that $\delta^{15}N$ in the forest floor, like N concentration and pool size, was constant from 1969 to 1992.

## Materials and Methods

### Site Description

This study was conducted on Watershed 6 (W6), the reference watershed at the HBEF in the White Mountains of New Hampshire (46°56′N, 71°45′W). The HBEF is a 3160-ha northern hardwood forest representative of much of the northeastern USA in stand age and disturbance history. The region was settled by Europeans in the late 1800s and selectively logged from about 1900 to 1917 (Whittaker et al., 1974). The dominant tree species are sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britton), with red spruce (*Picea rubens* Sarg.) and Balsam fir (*Abies balsamea* L. Mill.) at upper elevations. The bedrock is medium- to coarse-grain sillimanite schist of the Rangeley Formation. Soils are Typic, Aquic, and Lithic Haplorthods of the Tunbridge (coarse-loamy, isotic, frigid Typic Haplorthods), Lyman (loamy, isotic, frigid, Lithic Haplorthods) Berkshire (coarse-loamy, isotic, frigid Typic Haplorthods) Sunapee (coarse-loamy, isotic, frigid, Aquic Haplorthods) series, with minor inclusions of inceptisols of the Pillsbury (coarse-loamy, mixed, active, acid, frigid Aeric Epiaquepts) series, with little clay and a sandy loam texture (Lawrence et al., 1986; Johnson, 1991). Soils are generally acidic, with mineral soil pH values <4.5, and are ≈60 cm deep (Johnson, 1991), with a 3- to 15-cm forest floor (Likens et al., 1977; Huntington et al., 1988).

Mean annual atmospheric N deposition was 492 mol ha$^{-1}$ yr$^{-1}$ in bulk deposition and mean annual streamwater nitrate flux was 200 mol ha$^{-1}$ yr$^{-1}$ for the period 1964 to 1992 (Likens and Bormann, 1995).

Nitrogen fixation at the HBEF is ≈36 mol ha$^{-1}$ yr$^{-1}$ (Roskoski, 1980) and is negligible relative to plant uptake and internal N cycling. Denitrification is negligible at the HBEF (<100 mol ha$^{-1}$ yr$^{-1}$; Melillo et al., 1983). Nitrogen concentration is 2.06% in the Oie horizon and 1.36% in the Oa horizon (Huntington et al., 1988). The N pool is 32 kmol ha$^{-1}$ in the Oie horizon and 62 kmol ha$^{-1}$ in the Oa horizon (Huntington et al., 1988). The residence time for N in the O horizon is 12 to 15 yr (Gosz et al., 1976).

### Sample Collection

Forest-floor samples were obtained from material at the Hubbard Brook Sample Archive (Veen et al., 1994); 20 pits were chosen from the original pits in W6 in a stratified-random manner. The 1969 and 1978 archived samples were collected from Oi, Oe, and Oa horizons. In 1987 and 1992, the upper horizons were combined (Oie), and the Oa horizon was sampled as in previous years. Samples were collected using a 10 by 10 cm template in 1969 (Gosz et al., 1976) and a 15 by 15 cm template in subsequent years (Yanai et al., 1999).

Before 1981, the forest-floor horizons were designated L, F, and H (Federer, 1982) on the basis of visual determination of the stage of decomposition of organic matter. More recently (Soil Conservation Service, 1981) the forest-floor horizons were designated Oi, Oe, and Oa on the basis of percentage of rubbed fiber content. This semiquantitative method for separating the horizons is useful in the laboratory, but does not alter the visual horizon separation method used in the field at this site (Federer, 1982). Therefore, for this study, L is considered equivalent to Oi, F to Oe, and H to the Oa horizon. The one exception is the set of Oa (H) horizon samples that were collected in 1969. In that study, because Gosz et al. (1976) had made an effort to collect pure Oa horizon material, the samples did not include the lower and transitional portion of the Oa horizon where mineral content is higher. The result of this sampling approach is that the values obtained for loss on ignition (LOI) indicate a higher organic matter content for the 1969 Oa horizon samples than for Oa horizon samples collected in other years (Yanai et al., 1999).

To compare the Oie horizon data from 1987 and 1992 with the Oi and Oe horizon data from 1969 and 1978, it was necessary to calculate a weighted $\delta^{15}N$ value for the Oie for the two earlier years. Based on the 1969 measurements of Gosz et al. (1976), the N mass fraction of the Oi horizon was 15% and that of the Oe horizon was 85% of the total N in the Oie horizon.

### Sample Preparation and Analysis

Samples were pulverized in a shatterbox (SPEX Chemical and Sample Prep, model 8500, Metuchen, NJ), oven dried at 65°C, and loaded into tin capsules for N-isotope analysis. Isotopic analyses were performed using a Dumas combustion system in continuous-flow mode (Carlo Erba, Milan, Italy) followed by a VG Prism mass spectrometer (Laboratory 1: Harvard University) or using a Finnigan Delta-S mass spectrometer (Laboratory 2: Boston University Stable Isotope Laboratory). We report all isotope data as $\delta^{15}N$ values, which represent the per mil (‰) difference between the isotopic composition of the sample and that of atmospheric dinitrogen:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000 \qquad [1]$$

where $R_{sample}$ is the sample isotope ratio ($^{15}N/^{14}N$), and $R_{standard}$ is $^{15}N/^{14}N$ for atmospheric N$_2$, or 0.003 676 5.

Because samples were analyzed on two different instruments, we made thorough comparisons to ensure that the measurements were equivalent. Approximately 10% of the samples were analyzed in triplicate. The standard deviation of the triplicates was 0.13‰ at Laboratory 1, and 0.11‰ at Laboratory 2. The precision of the analysis for peptone standards in the N mass range of most of the samples was ±0.15‰ (SD) at Laboratory 1 and ±0.21‰ at Laboratory 2. Apple leaves from the National Institute of Standards and Technology (Standard Reference Material #1515) were run at Laboratory 2 only, and had a standard deviation of 0.20‰ and a mean of 0.35‰.

To verify the accuracy of the $^{15}N$ analysis at the two laboratories, we compared the mean values of peptone standards and of 25 samples that were run in both laboratories. The difference in peptone standard means between Laboratory 1
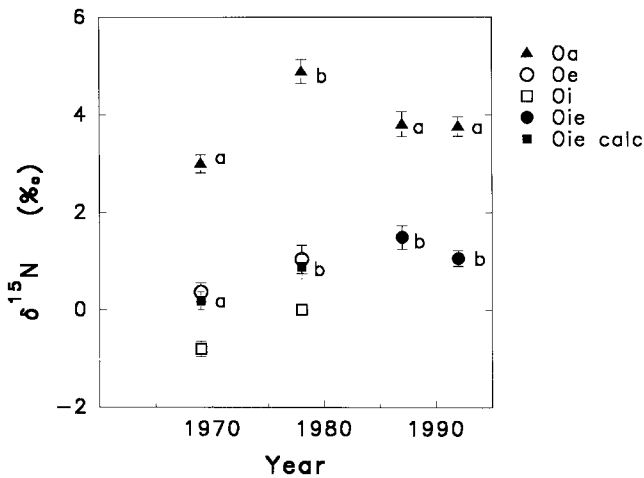
**Fig. 1. Natural abundance of ^{15}N in the forest floor of the reference watershed (W6) at the Hubbard Brook Experimental Forest. Natural abundance of ^{15}N for the Oa horizon is shown with filled triangles, for the Oe horizon with open circles, for the Oi horizon with open squares, and for the Oie horizon with filled circles. Calculated, mass-weighted mean values for the Oie horizon in 1969 and 1978 are shown with filled squares (see Materials and Methods section for calculation methods). Means within a horizon (Oa or Oie) associated with the same letter are not significantly different. Bars represent standard error ($n = 20$).**
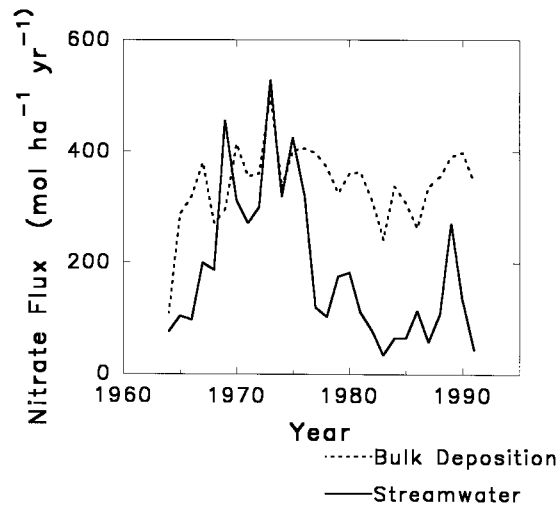


**Fig. 2. Nitrate bulk deposition inputs and streamwater outputs for the reference watershed (W6) at the Hubbard Brook Experimental Forest. Annual inputs of nitrate in bulk deposition (dotted line) and outputs of streamwater nitrate (solid line) for the reference watershed at Hubbard Brook Experimental Forest are shown for water years (June to May) from 1964 to 1992, after Likens and Bormann (1995).**

and Laboratory 2 was 0.12‰ and the mean difference for the 25 test samples was 0.01‰. These differences were considered negligible since they were not greater than the precision of analysis.

### Statistical Analysis

The effects of time and horizon were tested using ANOVA and compared using the Student-Newman-Keuls statistic at the $P < 0.05$ level (Montgomery, 1991). All statistical analyses were conducted using SAS (SAS Institute, 1988).

### Results

The $\delta^{15}N$ of the Oa horizon increased from 3.00‰ in 1969 to 4.89‰ in 1978 (Fig. 1); then decreased to 3.81‰ in 1987 and remained near that level in 1992. The $\delta^{15}N$ in the Oa horizon in 1978 was significantly higher than in all other years; there were no other significant differences ($P < 0.05$).

The calculated $\delta^{15}N$ for the Oie horizon was 0.17‰ in 1969; it increased to 0.91‰ in 1978 (Fig. 1) and remained at the higher level for the later years (1.49‰ in 1987 and 1.06‰ in 1992). The $\delta^{15}N$ in 1969 was significantly lower than in all other years; there were no other significant differences ($P < 0.05$).

To evaluate whether the difference between the 1969 and 1978 $\delta^{15}N$ in the Oa horizon was driven by low $\delta^{15}N$ values in 1969 caused by a different sampling method, we assessed the relationship between $\delta^{15}N$ and LOI. Loss on ignition was higher for the Oa horizon samples in 1969 than in other years; samples with a higher LOI contain less mineral soil. $\delta^{15}N$ is lower in the organic horizons than in the mineral soil (Fry, 1991; Nadelhoffer and Fry, 1994). However, in a pooled dataset from the

Oa horizon for 1969 and 1978, we found no correlation between LOI and $\delta^{15}N$. This suggests that the increase in $\delta^{15}N$ we observed was not an artifact of the different sampling methods.

### Discussion

The increase in $\delta^{15}N$ in both the Oie and Oa horizons follows a period from 1969 to 1976 of elevated streamwater nitrate loss from the W6 (Likens and Bormann, 1995; Fig. 2). During this period, the streamwater flux ranged from 275 to 550 mol ha$^{-1}$ yr$^{-1}$ compared with the mean of 200 mol ha$^{-1}$ yr$^{-1}$. Several scenarios have been proposed to explain these elevated streamwater nitrate losses (Goodale, 1999): (i) elevated N deposition (Likens and Bormann, 1995); (ii) a defoliating insect outbreak and a hailstorm in late summer 1969, which caused a significant loss of green foliage (Bormann and Likens, 1979); (iii) soil frost (Likens and Bormann, 1995); and (iv) climatic factors including temperature and precipitation patterns (Goodale, 1999). Of these explanations, the last explanation best accounts for similar, synchronous patterns of elevated N loss throughout the region (Goodale, 1999). Indeed, the PnET model accurately simulates the period of N loss when actual temperature and precipitation patterns are input to the model (Aber and Driscoll, 1997).

One scenario that would explain both the increase in nitrate loss and the observed increase in $\delta^{15}N$ in the Oa horizon would be a period of increased nitrification. Other possible explanations for the increase in $\delta^{15}N$ in the Oa horizon in 1978 include: (i) an increase in denitrification, or (ii) an increase in the amount and $\delta^{15}N$ of ammonium in atmospheric deposition retained by the forest floor. However, neither of these explains the

streamwater nitrate pattern (Fig. 2). Moreover, even assuming complete retention of atmospherically deposited ammonium with the highest reported $\delta^{15}N$ value, an increase in forest-floor $\delta^{15}N$ of the magnitude measured in 9 yr would not have been possible. Streamwater nitrate losses decreased after the mid 1970s, and $\delta^{15}N$ in the Oa horizon returned to its 1969 value.

Nitrogen cycling in the Oie horizon differs from that in the Oa horizon because of large inputs of aboveground litter and significant immobilization of N in the Oie horizon compared with the Oa horizon (Aber and Melillo, 1980). As in the Oa horizon, if nitrification and nitrate loss were to increase, the ammonium pool would become enriched in $^{15}N$ (Handley and Raven, 1992; Nadelhoffer and Fry, 1994); if that ammonium were retained in the Oie horizon, the total Oie would become enriched in $^{15}N$. Moreover, we expect that with the leaching of $^{15}N$-depleted nitrate and uptake of enriched ammonium, plants and thus any subsequent litterfall would become enriched in $\delta^{15}N$, as we observed following clear-cutting at the HBEF (Pardo, 1999).

It may be significant that, in contrast with the Oa horizon where $\delta^{15}N$ increased and then decreased, $\delta^{15}N$ in the Oie horizon remained elevated. If the increase were due to elevated nitrification and nitrate loss that then returned to lower levels, ammonium retained in the Oie horizon would no longer be enriched in $^{15}N$. However, in contrast to the Oa horizon, the enrichment of the Oie horizon via aboveground litter inputs may delay recovery. The $^{15}N$-enriched litter inputs could enrich the organic-matter N pool itself. This enrichment effect may be more long lasting compared with retention of enriched exchangeable ammonium. Enriched organic matter from the Oie horizon could exert a slight influence over time on the Oa horizon $\delta^{15}N$. However, the enrichment of the Oie would need to be substantial (since it has a lower $\delta^{15}N$ value than the Oa horizon to begin with) and of a duration longer than the turnover time of the Oa horizon. Neither of these conditions was met during this time period at the HBEF.

## Conclusions

Natural abundance of $^{15}N$ was not at steady state in either the Oie or Oa horizon from 1969 to 1992 in the W6 at the HBEF. The increase in Oie- and Oa-horizon $\delta^{15}N$ values following a period of elevated streamwater nitrate loss suggests that elevated nitrification in the forest floor may have been the source of increased streamwater nitrate. The increase in $\delta^{15}N$ in the Oa horizon was larger than that in the Oie horizon, but the increase in $\delta^{15}N$ in the Oie horizon beginning in 1978 persisted for the rest of the period of record. Differences in patterns of $\delta^{15}N$ with time in the Oie compared with the Oa horizon suggest that different factors regulate N cycling, and therefore the $\delta^{15}N$, in each horizon.

When nitrate losses were high, $\delta^{15}N$ in the forest floor increased. This pattern suggests that disruptions of the N cycle (either anthropogenic or natural disturbances)

can alter $\delta^{15}N$ and should be considered in evaluating natural abundance observations. Finally, these stable isotope data provide some information about a puzzling period in N dynamics at the HBEF (1969–1976). Goodale (1999) suggests that the period of elevated N losses was caused by climatic factors including temperature and precipitation patterns that occurred throughout the region. While the data from this study neither support nor refute this explanation, they do suggest that nitrification increased during the period of high nitrate losses.

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